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Nature of Papain

by

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THE NATURE OF PAPAÏN AND ITS ACTION ON VEGETABLE PROTEID[1]. By SIDNEY H. C. MARTIN, M.D. (*Lond.*) B.Sc. M.R.C.P. *Fellow of University College, Lond.*

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In a previous paper[2] I have detailed the action of the proteolytic ferment papaïn on coagulated egg-albumen and on fibrin, and have shown by extending the researches of Wurtz, Bouchut, Albrecht and others that papaïn acts like trypsin. The results obtained may be summarised as follows:—

The ferment acts best in alkaline solutions of the strength of $\frac{1}{4}$ p.c. sodium carbonate: but it acts well in neutral media.

It acts less energetically than trypsin, but the products formed are the same, viz., a perfect peptone, an "intermediate" body of the nature of a globulin, and leucin and tyrosin—the last being in only small quantity.

In the present investigation I have extended my observations into the nature of the ferment itself, the characters of the proteids contained in papaw juice, and the action of papaïn on these. I will therefore consider the subject under two heads:

(1) Nature of the ferment.

(2) Proteolytic changes in the proteids of papaw juice.

PART I.

Nature of the Ferment.

In the commercial papaïn I used in my first experiments I found two soluble proteids, which as I have stated[3] were a globulin and a peptone, the latter disappearing to some extent by dialysis. I had not

[1] Towards defraying the expenses of this investigation a grant was obtained from he Scientific Grants Committee of the British Medical Association. A short summary appeared in the *Brit. Med. Journ.* July, 1885.

[2] This *Journal*, Vol. v. No. 4.

[3] *Loc. cit.* p. 215.

at that time come to any conclusion as to which of those bodies was the ferment, or, to speak more correctly, with which the ferment-action was associated. I thought that it was not the globulin, chiefly from the reactions which Wurtz[1] had given as belonging to the solution of a pure and active papaïn. He found that this body was a proteid, not precipitated by boiling or corrosive sublimate, but giving precipitates with nitric and hydrochloric acids soluble in excess, and one also with acetic acid and potassic ferrocyanide. These reactions indicate a body, not a native albumen, not a globulin, not a peptone, but one of those bodies which Kühne[2] has described under the name of albumose, to the consideration of which we shall presently come.

If the body be an albumose, it must be present in the active "papaïn" I used; and this I found to be the case—the "peptone" I had previously described being not a true peptone, but a hemialbumose.

To demonstrate this the following methods were adopted:

Method A. One gramme of an active papaïn was extracted for 24 hours with pure glycerine, and then filtered under vacuum pressure. The filtrate was very slightly opalescent, and on dilution gave the following reactions:

1. A faint cloudiness on boiling, soluble in nitric acid, and giving a marked xanthoprotein reaction.

2. An abundant precipitate with nitric acid, almost completely soluble on boiling, coming down again on cooling, redissolving on boiling etc.—a reaction characteristic of an albumose.

3. A biuret reaction with copper sulphate and excess of potash.

The body then extracted by glycerine was chiefly an albumose.

To see whether any globulin was present in the glycerine, this was diluted, the solution being neutral, and saturated with magnesium sulphate, causing only a trace of precipitate, which was filtered off. The filtrate gave the reactions of hemialbumose, and was then saturated with sodium sulphate and shaken for one hour; the double saturation caused a dense precipitate, which was collected on a filter, washed with a saturated solution of sodio-magnesium sulphate and dissolved by adding distilled water. The activity of this solution of hemialbumose was tested. The filtrate after double saturation gave scarcely any xanthoprotein reaction, showing that most of the proteid had been precipitated: it was dialysed in running water for 19¼ hours and its activity tested.

[1] *Compt. Rend.* June, 1880.

[2] Kühne and Chittenden, "Ueber Albumosen," *Zeits. für Biologie*, Bd. xx.

The results obtained may be stated as follows:

1. *Glycerine extract of papaïn*, containing chiefly hemialbumose with a trace of globulin. In a neutral medium this forms peptones in quantity from coagulated egg-albumin, in 24 hours' digestion at 32° C., the globulin-like body being also formed—7 grms. of moist albumin leaving about 2 grms. residue. The extract is therefore active.

2. *Solution of albumose*, obtained by precipitation with sodio-magnesium sulphate from the diluted glycerine extract. A very small quantity (not weighed) with 10 grms. of coagulated egg-albumin and 100 cc. of water formed peptones in abundance in 19¼ hours at 35°—40° C., the intermediate body also appearing. The action then is the same as that of the glycerine extract.

3. *Dialysed filtrate*, after double saturation of glycerine extract, containing a trace of hemialbumose. In 27 hours' digestion at 33°—35° C. with 10 grms. of coagulated albumin in 100 cc. of water, no intermediate body was formed, and only a faint cloudiness could be obtained with acetic acid and metatungstic acid, showing indeed only a trace of peptones.

Comparing these results, it will be evident that where there is albumose there is a ferment-action, and that this union is so close that the proteid precipitated by the double neutral salt (sodio-magnesium sulphate) is an active proteolytic.

A similar method of saturation with salts was carried out in a watery solution of the same commercial papaïn with the following results:

The neutral solution was first saturated with magnesium sulphate giving a precipitate of globulin; this was collected, dissolved by adding distilled water, dialysed for 72 hours (with thymol), and used for digestion. The filtrate after the first saturation was shaken with sodio-magnesium sulphate for some hours, causing a precipitate, which after solution in distilled water was dialysed for 3 days; this precipitate gave the reactions of albumose and also of a trace of globulin. The last filtrate, after dialysis for 5 days, gave only a slight xanthoprotein reaction.

The activity of these, the globulin, the albumose, and the filtrate were tested, and the results may be stated as follows:

Magnesium sulphate precipitate—*globulin*. No action on uncoagulated egg-albumen in neutral medium at 35°—40° C.

Sodio-magnesium sulphate precipitate—*chiefly albumose*, trace of globulin. Formed peptones from egg-albumin (diluted) at the same temperature.

Filtrate with only a trace of proteid. No action on egg-albumin.

The two experiments just quoted prove definitely the association of the ferment action with the albumose present. Another experiment was however performed in order to isolate the ferment more completely than can be done by saturation with salts, owing to the difficulty of removing all the latter by dialysis.

Method B. A large excess of alcohol (86%) was added to 1 gramme of papaïn and the mixture allowed to stand 14 days to coagulate the globulin. The liquid was then filtered, the residue dried and extracted for 48 hours with pure glycerine; the extract was filtered under vacuum pressure and allowed to drop into a mixture of absolute alcohol, 8 parts, pure sulphuric ether, 1 part[1]. This caused a dense flocculent precipitate, which was caught on a filter, and dissolved, in part in glycerine, and the remainder in distilled water. These solutions gave the reactions of albumose only, there being no precipitate by boiling, a dense one with nitric acid, and a biuret reaction with copper sulphate and potash. Both the first and second glycerine extracts and the watery solution of the albumose were actively proteolytic, forming peptones from coagulated egg-albumin.

To complete the experiment, the mixture of alcohol, ether, and glycerine was allowed to evaporate in the top of an incubator at 35° C. till the two first had been dissipated, and the activity of the glycerine residue was tested on milk and coagulated egg-albumin. It was found to have no action whatever, neither peptones nor the intermediate body being formed: in the experiment, the second glycerine extract formed peptones both from milk and coagulated albumin. Alcohol therefore precipitates all the ferment from a glycerine solution.

From these experiments there is no doubt that papaïn is associated with an albumose; and to the reactions previously stated by Wurtz, I would add the following:

1. Solutions give a biuret reaction.

2. The body is precipitated from a neutral solution by saturation with sodio-magnesium sulphate, the precipitate being still active. It is not precipitated by magnesium sulphate saturation alone, or by sodium chloride, except in acid solution.

3. It is dissolved by glycerine, and if precipitated from this solution by alcohol, the filtrate has no proteolytic power.

The question still remains as to which particular kind of albumose

[1] This was the method adopted by Gorup-Besanez in extracting the proteolytic ferment from vetches: *Ber. d. deutsch. chem. Gesellsch.* VII. 1874.

this proteid is. Kühne and Chittenden[1] have described four varieties, differing chiefly in their solubility in distilled water and salt solution, and in their precipitation by saturation with sodium chloride. These varieties are called prot-, hetero-, dys-, and deuteroalbumose.

Putting aside the question whether these are all distinct bodies, or only modifications of one or two, the albumose described above would correspond most nearly to protalbumose, being soluble in distilled water and precipitated by nitric acid. It however differs in one important particular, viz. in not being precipitated by saturation with sodium chloride in neutral solution, but only when this is distinctly acid: hence it is not the form of albumose described by Kühne and Chittenden. The more complete treatment of this point will be left till the proteids of the papaw juice are discussed: at present, it may be stated that it is the same body described later as *a-phytalbumose.*

Besides the proteolytic ferment, there is also a *milk-curdling ferment,* which I have not yet succeeded in isolating from the former. The powder itself (commercial papaïn) curdles much more actively than the glycerine extract, though the latter does possess the property, even after precipitation by alcohol and ether (as in B, detailed above).

The following results of experiments show the activity of this curdling:

Papaïn (·3 gramme) in 450 cc. of milk diluted with 125 cc. of water caused curdling at once, at 62° C.

Papaïn (·5 gramme) in 200 cc. of milk and 50 cc. of water, with 1 gramme of sodic bicarbonate added, caused curdling in 5 minutes at 60° C. A greater excess of the alkaline carbonate and a lower temperature delays the curdling; thus with 200 cc. of milk, ·5 gramme of papaïn, and ·5 gramme of sodic carbonate, at 43° C., curdling occurred in 15 minutes. Also, diluting the milk hinders the curdling: in a similar experiment, e.g., in which 200 cc. of milk were diluted with an equal volume of water, the same quantity of papaïn and alkali being added, curdling was delayed (at 48° C.) for $1\frac{1}{4}$ hour, yet the ferment was active, since peptones and the intermediate body were formed.

The curdling is a precursor of digestion, but its relation to the transformation of the casein into peptones I have not yet determined, since it depends on the separation of the proteolytic, and the curdling, ferments—a result not yet accomplished. It is probable that the two ferments are distinct, judging from the analogous association in

[1] *Op. cit.* p. 17.

pancreatic juice: Dr W. Roberts of Manchester has in this case been able to separate them[1].

Baginsky[2] has investigated the curdling ferment in papaw juice: he appears to think that sodic carbonate has no effect in hindering the curdling, though only one experiment is quoted[3].

There is no diastatic ferment associated with papaïn: it has no action whatever on starch in dilute solution.

PART II.

Proteolytic changes in the proteids of papaw juice.

Before dealing with the kind and character of proteids present in the juice, a short account of the present state of knowledge as regards vegetable proteids is necessary.

Speaking generally, the chief proteids present in seeds have been determined by Hoppe-Seyler[4], Weyl[5], and Vines to consist of globulins and peptone. The globulins according to Weyl are of two kinds, resembling animal myosin and vitellin; the peptone is considered by Vines to be a hemialbumose. There is no doubt whatever from the experiments quoted by these observers that these two groups of bodies are present. It still remains doubtful, however, whether a true peptone is not present: viz. a proteid, soluble in distilled water, not precipitated by nitric acid or acetic acid and potassic ferrocyanide, and giving a biuret reaction. It is doubtful because though Vines[6] separated a body from the seeds of *Lupinus varius, Paeonia officinalis, Ricinus communis,* and other plants, giving the reactions of hemialbumose, yet there may be a true peptone present as well: he does not mention experiments separating the former from the solution and seeing whether any proteid remained. This is a point of some importance, and to it I shall return.

The question as to whether a native albumin (corresponding, e.g. to white of egg) is present in plants is still unsettled, though it is decided in the affirmative by Ritthausen[7].

[1] Cromian Lectures on Digestive Ferments.

[2] *Zeits. für physiolog. Chemie*, Band. VII. 1882.

[3] A more detailed account of the action of papaïn on milk will be found in my Report in the *Brit. Med. Journ.* July 25, 1885.

[4] *Physiologische chemie.* [5] *Zeits. für physiolog. Chemie*, Bd. II. 1877.

[6] *Proc. Roy. Soc.*, vol. XXVIII. 1878. *Ibid.* vol. XXX. *Ibid.* 1880. *Journal of Physiology.* "Proteid substances in seeds." Vol. III.

[7] *Die Eiweisskörper der Getreidearten, Hülsenfrüchte und Oelsamen.* Bonn, 1872.

Further the extensive researches of this observer, which have been almost universally accepted (even up to 1877 by Sachsse[1]), discovered the presence of bodies which he called legumin, conglutin and gluten-casein. Vines has explained the presence of the two first bodies by an action of the alkali (used in extracting them) on the globulins present; the last being also probably due to the same cause.

Weyl considers all the caseins described in plants as artificial products.

The question cannot be further discussed here: the whole subject has been ably treated by Vines in this journal[2].

The important fact brought out by the researches of Weyl, Hoppe-Seyler, Zoller, supplemented by those of Vines, is that there is a constant association of globulins and hemialbumose in the seeds of the plants investigated. This fact will derive still greater significance from the results of the analysis of the proteids in papaw juice, immediately to be detailed.

As mentioned above it does not seem to me definitely proved that true peptones are absent from the vegetable kingdom: and to this point I have directed my attention specially. To return to what has previously been said, the hemialbumose described by Vines was, like peptone, soluble in distilled water, and also, like that body, was not precipitated by boiling, and gave a biuret reaction: it differed from peptone in being precipitated by nitric acid and acetic acid and potassic ferrocyanide, and also, according to Vines, in not dialysing. It seemed to me therefore necessary to use a method which would separate the hemialbumose, leaving the peptone, if present, in solution. Several methods were attempted, the readiest of which was found to be Hofmeister's. It is as follows: to the liquid, which must be neutral, is added half its volume of a saturated solution of sodium acetate, and then dilute ferric chloride till no more blood-red colouration is observed: ferric acetate is formed. The liquid is then neutralised with caustic soda, care being taken that it is on the acid rather than on the alkaline side of neutrality, boiled, and afterwards filtered. The clear filtrate is free from all proteids except peptones.

I have used this test in the digestive filtrates of both pancreatic and papaïn digestions and have found it answer well. In the filtrate there will be no precipitate with nitric acid or with acetic acid and potassic ferrocyanide, showing the absence of hemialbumose; and yet there will

[1] *Die Farbstoffe, Kohlehydrate, und Proteïnsubstanzen.* Leipzig, 1877.

[2] *Op. cit.*

be an intense xanthoprotein and a biuret reaction with copper sulphate and caustic potash, if peptones are present in quantity. If these are only small in amount, no biuret reaction will be obtained, though still the xanthoprotein: their presence is then best demonstrated by adding one-fifth volume of acetic acid and a drop or two of acid phosphotungstate of soda (metatungstic acid), when on standing for some minutes a cloudiness will appear, settling to a fine precipitate.

This last test was much used by Poehl[1] in his investigation, both for the detection and the quantitative estimation of peptones. I found as the result of many experiments that some forms of albumose were with difficulty precipitated by boiling with ferric acetate, so that the operation had to be repeated with the filtrate: and it may moreover be stated here, that a body resembling Meissner's b-peptone, to be described later, is not precipitated, as I have found, in this way. A small quantity of peptone is also precipitated, as may be deduced from the fact that a solution containing no proteid but peptone gave a fainter biuret and less precipitate with acetic acid and metatungstic acid after boiling with ferric acetate than before. The quantity thrown down however is small, and may be recovered by shaking the precipitate with water[2].

Another important property of peptones, which has been utilised in the present investigation, is that they are not precipitated by double saturation with magnesium and sodium sulphates, or by the double salt sodio-magnesium sulphate. This was tested several times in a solution of peptones in which neither boiling, nitric acid, nor acetic acid and potassic ferrocyanide gave any precipitate; saturation with sodio-magnesium sulphate (with 1 hour's shaking) gave no precipitate, and the filtrate gave a cloudiness passing on to a dense precipitate with acetic acid and metatungstic acid. This brings out another point, viz. that the last reaction for peptones can be performed in a saturated solution of salts: the biuret reaction not being obtainable because of the precipitation of the magnesium by the alkali.

The importance of this non-precipitation of peptones by sodio-magnesium sulphate will appear afterwards.

Lastly, it may be stated that peptones are precipitated by saturation with ammonium sulphate. I can, in this matter, confirm the observations of Heynsius[3].

Bearing in mind the researches previously mentioned, I directed my

[1] *Ueber das Vorkommen u. die Bildung des Peptons etc.* Dorpat, 1882.

[2] Poehl, *op. cit.* [3] Heynsius, *Pflüger's Archiv*, Bd. 34, S. 330.

attention chiefly to the detection of globulins and hemialbumose in papaw juice, at the same time seeing whether any native albumin or peptone were present.

The material[1] used was the juice of the unripe fruit, dried (chiefly in the East Indies) in the open air and under glass. In this form, it is a yellow brown powder of sickly smell.

The methods used in obtaining the proteids from this dried juice were two; one consisted in extracting for some hours with a 10 to 15 % sodium chloride solution, the other in using simply distilled water. By the former most of the proteids would be dissolved, globulins, albumoses, and albumin (if present): by the latter, it was thought that much of the globulin might remain undissolved, the solution consisting chiefly of the other proteids present.

We shall deal with the watery extract first.

Experiment. *July* 13, 1885. A specimen of artificially dried fruit milk was extracted with distilled water, being filtered after shaking by the hand. The filtrate was dark brown and distinctly acid. Neutralisation caused a slight precipitate, soluble in excess of alkali: there was therefore some acid-albumin present[2]. After reprecipitation, the solution was filtered, and the neutral filtrate gave the following reactions:—

1. Boiling, a dense precipitate, which was filtered off. The filtrate at first clear became turbid on cooling: it was returned till quite clear.

(α) The precipitate, after being well washed with water, was treated with ·2% H^2SO_4 in which the greater part dissolved. The acid solution gave a precipitate on neutralisation, a very faint biuret reaction, and a precipitate with nitric acid, soluble on warming, coming down again on cooling, redissolving on heat: behaving in fact like an albumose. The solution also gave a marked xanthoprotein reaction.

The precipitate was also soluble in ·2% KHO, giving similar reactions.

(β) The filtrate gave no precipitate on boiling, but a dense one on adding nitric acid, soluble in excess, and on heating, coming down again on cooling etc.; also precipitates with mercuric chloride and basic acetate of lead, the former insoluble, the latter soluble in excess of the reagent; also a biuret reaction. Saturation with sodium chloride in neutral solution caused no precipitate, but one immediately appeared when the liquid was made slightly acid and shaken with more salt.

[1] For many valuable specimens I am indebted to the kindness of Messrs Christy & Co. of Fenchurch St.

[2] The acidity of the solution of the dried juice and the presence of acid albumin must be considered artificial: since the fresh juice is neutral in reaction. The dried juice used had been kept for many months.

2. After boiling with ferric acetate, the filtrate gave no biuret reaction, and no precipitate with acetic acid and metatungstic acid: showing the absence of peptones.

This experiment shows that water extracts from the juice, (1) proteids which are precipitated by boiling; the precipitate however did not wholly consist of coagulated proteid, since it was partly soluble in dilute acid and alkali, the solutions giving the reactions of an albumose, as shown by the characteristic behaviour of the nitric acid precipitate and by the biuret reaction: (2) a proteid not thrown down by boiling, but by nitric acid, the precipitate behaving like an albumose. It is this proteid, indeed, that is called by Vines hemi-albumose, and which previous to his observations was called vegetable "peptone."

It agrees with Vines' hemi-albumose in not being precipitated by saturation with sodium chloride[1], though it is thrown down if the solution be distinctly acid; it differs in not being precipitated by acetic acid[2]. Its solubility in water, its non-precipitation by boiling and its reactions with basic acetate of lead and mercuric chloride bring it into relation with Kühne and Chittenden's prot-albumose: from which it materially differs in its non-precipitation by saturation with sodium chloride in neutral solution. It will be noticed that it is the same body as that with which the ferment action is so closely associated. Provisionally I shall propose, at the suggestion of Professor Schäfer, to call this body *α-phytalbumose*, in distinction to another form (β) to be described later.

The separation in an unchanged form of the bodies precipitated by heat was found more difficult. The coagulated portion of the precipitate may consist either of globulins or an albumin, or both: the part soluble in dilute acid and alkali consists of an albumose.

From the watery extract, only the separation of the albumin was attempted, the other two bodies being more readily obtainable from the saline extract.

Separation of the albumin.

The watery extract, freshly made, was saturated, after neutralisation and filtration, with sodium chloride, causing a dense precipitate, which when dissolved was found to consist of coagulable proteid in small amount, and an albumose, in quantity.

Since the *α*-phytalbumose was not thrown down (the solution being

[1] *Proc. Roy. Soc.*, Vol. XXVIII. 1878. [2] *Ibid.* Vol. XXX. p. 387.

neutral) these bodies were presumably the same as those previously indicated. The filtrate was then made distinctly acid (with acetic) and shaken with more sodium chloride, causing a dense precipitate which was filtered off: this consisted of *a*-phytalbumose and some of the two proteids unprecipitated by the previous saturation. As the filtrate still gave a precipitate on boiling it was dialysed for 3 days (with addition of thymol) in running water: after dialysis, the salt had disappeared, as the liquid gave no precipitate with silver nitrate: barium chloride also gave no precipitate. There was no turbidity caused by dialysis, showing that no globulin (vitellin) was present, since this body is precipitated by dialysis. Moreover the almost complete removal of albumose was confirmed by saturation with sodium chloride in acid solution causing only a slight cloudiness. The solution after dialysis gave the following reactions:

1. A dense precipitate on boiling, insoluble in nitric acid, weak or concentrated, or in ·2% H^2SO_4 and ·2% KHO, but slightly soluble in strong potash. The precipitate was therefore coagulated proteid. The filtrate after boiling contained a trace of *a*-phytalbumose.

2. A dense precipitate with nitric acid, only very partially soluble on heating, coming down again on cooling etc. a reaction indicating the presence of some albumose; an intense xanthoprotein reaction on the addition of ammonia.

3. A precipitate with basic acetate of lead, insoluble in excess, and a biuret reaction (due to the albumose) with copper sulphate and potash.

4. After saturating with sodium chloride and filtering off the slight turbidity, the clear filtrate gave a dense precipitate on boiling and with nitric acid.

We have then distinct evidence of a body belonging to the class of native albumins, since its solutions are not precipitated by dialysis or by saturation with sodium chloride, but are coagulated by heat and nitric acid.

The experiment also shows that there is no vegetable vitellin present[1].

To sum up the results obtained from the analysis of the watery extract of the juice, we have certain evidence of the presence of a native albumin and an albumose, called above *a*-phytalbumose; and an

[1] It was erroneously stated in a short report of this investigation in the *Brit. Med. Journal*, July, 1885, that a trace of vitellin was present. From later experiments I am convinced that such is not the case.

indication of the presence of a globulin and of another albumose, both precipitated by heat and by saturation with sodium chloride.

There is no vegetable vitellin present, and no peptone.

It now remains to enter more fully into the separation of the globulin and albumose. Their behaviour to heat and saturation with sodium chloride are, as we have seen, the same. It was thought possible that there might be a difference in their behaviour to saturation with magnesium sulphate, to dialysis, and in the precise temperature at which they were precipitated by heat.

Precipitation of saline extract by saturation with magnesium sulphate in neutral solution.

A large excess of 10% solution of sodium chloride was shaken with a specimen of dried fruit milk and filtered immediately. The dark brown filtrate was clear and acid. It was neutralised with caustic soda, causing a precipitate of albuminate, as in the watery extract. This was filtered off and the neutral filtrate shaken with magnesium sulphate by the hand, causing a dense precipitate, which was caught on the filter. After washing with a saturated solution of the salt, the precipitate was dissolved completely by adding distilled water and the clear brown solution dialysed for 3 days. Presumably then we have here a precipitate consisting chiefly of globulin, but also of some β-phytalbumose, the α-phytalbumose not being thrown down because the solution was neutral; and this was confirmed by the fact that boiling and filtration removed nearly the whole of the proteid present, the small amount in the filtrate being due to the fact, as we shall see, that all the β-phytalbumose is not thrown down by boiling.

Partial removal of the salt from the solution caused a fine precipitate, which was caught on a filter, washed with distilled water, and then dissolved readily in 10% NaCl. This solution gave a precipitate on boiling insoluble in dilute acid, and a precipitate with nitric acid, quite insoluble on boiling, showing the presence of a coagulable proteid, a globulin.

The coagulation point of this globulin was tested by gradually heating in a test tube placed in a flask containing water. The solution began to get cloudy at 70°C., the cloudiness increasing to 73°; kept for a short time at this temperature, a fine precipitate fell to the bottom of the tube.

A fractional precipitation by heat was performed in the filtered dialysed liquid, which presumably still held some globulin in solution. It became cloudy at 70°, a fine precipitate falling as before at 73°. The filtered liquid became cloudy again at 78°, the cloudiness increasing to 84°, when a precipitate fell: the liquid was not heated further. The precipitate falling between 70° and 73° was the globulin, that between 78° and 84° was the albumose, the peculiar behaviour of which to heat will be noticed more fully afterwards.

These results are with advantage compared with the fractional precipitation by heat performed in the 10% NaCl extract, to which we shall soon come: they show that the globulin and albumose are precipitated at different temperatures.

The magnesium sulphate precipitate was dialysed till potash gave no jelly precipitate; silver nitrate also gave none, but barium chloride gave a distinct cloudiness, showing the presence of sulphates in small amount.

The liquid was turbid with more precipitate (globulin), so this was filtered off. The clear filtrate gave a dense precipitate on boiling, soluble in dilute acid and alkali. This precipitate then consisted of albumose. Nearly all the globulin had been removed, as shown by the almost complete solubility of the nitric acid precipitate in boiling.

The properties of this albumose were then investigated.

(*a*) As before stated it is precipitated by boiling. The extent of this precipitation was tested; thus after boiling in neutral solution, and filtering, the filtrate became cloudy on falling into the cool test-tube, and when returned till clear was found still to give a cloudiness on heating; this was again filtered off, when boiling produced no further cloudiness in the filtrate, and nitric acid only a slight cloudiness.

The incomplete precipitation of the body by the first boiling, and the fact that the clear hot filtrate becomes turbid on cooling, shows that the albumose is slightly soluble at a boiling temperature, and is re-precipitated by cooling: further, this reaction explains the peculiar behaviour of the filtrate of the boiled watery and saline extract of the juice.

The precipitate obtained by boiling the solution of the albumin is not coagulated proteid in the ordinary sense of the term. It is insoluble in 10% magnesium sulphate or sodium chloride solution, but is soluble in ·2% sulphuric acid, and in ·2% potash solution, more readily in the former than in the latter.

The solution in the dilute acid is not acid albumin, since nitric acid

gives a precipitate soluble on heating, coming down again on cooling, redissolving on heating etc.: the precipitated proteid then retains the character of an albumose. It is not precipitated from the acid solution by boiling, but comes down on neutralisation, the precipitate being soluble in excess. Moreover, with excess of potash and a trace of copper sulphate, it gives a biuret reaction. In this solution then it resembles acid-albumin in not being precipitated by boiling, and in the fact that the neutralisation precipitate is insoluble in water, but soluble in excess of alkali. The solution of the precipitated albumose in ·2% potash solution behaves in a similar manner to the acid solution.

Precipitation temperature of β-phytalbumose. From many experiments it was found that the solution became cloudy at 78° C., the cloudiness increasing to 82° C., at which temperature a precipitate fell in a minute or two. This precipitate was filtered off; the filtrate again heated became cloudy at 83° C. (85° C.) depositing a flocculent precipitate at 92° C. (95°C.); the liquid was raised to the boiling point and filtered. The filtrate still gave a cloudiness on boiling, showing, as was before seen, the difficulty with which this body is precipitated by heat.

The precipitates which fell at 78°—82° C. and 85°—95° C. (83°—92° C.) were both soluble in dilute acid and alkali, they were both in fact the precipitated albumose. The behaviour of this body to heating is then peculiar and unlike any other precipitable proteid yet known.

Moreover, if the liquid be made acid, so that it just gives a reaction with delicate test paper, the albumin is very imperfectly precipitated from solution, and the point at which this precipitation occurs is raised, a cloudiness not appearing till 90° C., increasing to 98° C. If slightly more acid be added, no precipitate is found at all.

(β) The albumose in solution is readily precipitated by nitric and hydrochloric acids, but not by glacial acetic acid unless potassic ferrocyanide be added. The nitric acid precipitate is soluble in excess, and on boiling, provided the heating be gradual: it reappears on cooling, redissolves on heating etc. The precipitate becomes less and less soluble on heating, the more sodium chloride or magnesium sulphate is present—a fact emphasized by Kühne and Chittenden as a property of the albumoses they investigated. The nitric acid precipitate if removed by filtration is readily soluble on adding distilled water: it gives a well-marked xanthroprotein reaction.

(γ) Its solutions give a precipitate with basic acetate of lead,

insoluble in excess. No precipitate falls on adding mercuric chloride, even if the solution be made acid, and none could be obtained with copper sulphate: it gives a faint biuret reaction.

(δ) As has been seen, saturation with sodium chloride causes a ready and copious precipitate in neutral solution, complete if this be made slightly acid. Magnesium sulphate to saturation precipitates the albumose less completely.

Dialysis, continued till all magnesium and chlorides are removed and only a trace of sulphates is present, causes no precipitate.

(ε) *Behaviour of the albumose to dilute acid and alkali.* The solution made slightly acid with dilute sulphuric acid may be heated gradually until boiled without causing any precipitate. This we have before seen. Neutralisation of the acid liquid causes a precipitate, insoluble in distilled water, and in 10% NaCl solution, but readily soluble in ·2% KHO and ·2% H^2SO_4. As regards solubility then this neutralisation precipitate behaves very much like the albumose when precipitated by heat: its resemblance to an albuminate will be noticed. Neutralisation however does not precipitate all the albumose, since the filtrate gives a marked cloudiness on boiling, and a precipitate with nitric acid behaves like an albumose. From these results we may conclude that dilute acids change the albumose in part into a body resembling an albuminate and the coagulated albumose; and also that the body is in part unaffected, as shown by the reactions of the filtrate after neutralisation.

Dilute alkali has still less action on the albumose. However gradual the heating may be, in a flame or in an incubator at 35°—40° C. for 24 hours, neutralisation causes only a slight precipitate soluble in excess of acid, and the filtrate gives reactions exactly the same as before the action of the alkali.

Fractional precipitation by magnesium sulphate and sodium sulphate in acid solution.

We have seen from the experiment just quoted that the globulin and β-phytalbumose are precipitated by saturation with magnesium sulphate in neutral solution—the former completely, the latter incompletely. We have moreover seen from previous experiments that α-phytalbumose is not precipitated in neutral solution, but only in acid.

In the present experiment, therefore, we should expect the magnesium sulphate precipitate to consist of globulin and the two albu-

moses. This was found to be the case. A 15% NaCl extract of the dried juice was saturated (while acid) with magnesium sulphate, being shaken for 4 hours on an engine: the dense precipitate was removed. Further shaking for periods of 2 and $3\frac{1}{2}$ hours also caused precipitates which were filtered off. The several precipitates were washed with a saturated solution of magnesium sulphate, and dissolved by adding distilled water.

The last filtrate after saturating with magnesium sulphate was shaken with sodium sulphate, causing a slight precipitate, which was washed with a saturated solution of sodio-magnesium sulphate, and dissolved by adding water.

After saturation with sodium sulphate, the filtrate gave no precipitate on boiling, or with nitric acid: and hence contained no proteid, precipitable by the neutral salts used; it was therefore examined for peptones.

1. The magnesium sulphate precipitate was found to consist of the globulin and the two albumoses. After dialysing the solution for 5 days in running water, the globulin was precipitated, and the albumoses remained in solution, giving the reactions previously detailed as characteristic of them.

2. The sodio-magnesium precipitate consisted almost wholly of *a*-phytalbumose, there being a trace of coagulable proteid present, which was presumably the albumin previously described. It was in too small quantity to decide as to its character.

3. The filtrate, as before stated, gave no precipitate with boiling or nitric acid. Boiled with ferric acetate in neutral solution, the filtrate gave no biuret reaction and no precipitate with acetic acid and metatungstic acid, showing the absence of peptones.

Fractional precipitation then by these salts, viz. saturation with magnesium sulphate, followed by sodium sulphate, does not in this instance teach us more than simple saturation with magnesium sulphate; since, as is seen, this salt precipitates the globulin and albumoses present, leaving a small quantity of *a*-phytalbumose in solution; this was readily thrown down on shaking with sodium sulphate. If any vitellin or albumin were present they would also be in the sodium sulphate precipitate.

This method of precipitating a mixture of proteids in an unchanged form from solution, first performed by Professor Schäfer[1], is one of the

[1] This *Journal*, Vol. III, p. 184.

most valuable in analysis. It has been used both by Schäfer and Halliburton[1] for separating the proteids of serum—which consist speaking broadly of albumen and globulin; it may be extended to the separation of albumoses.

It is not proper to generalise from the analysis of only one mixture of plant proteids, but the following extended table may be given for the guidance of future researches, which alone can decide how far the existence of the albumoses described are general in the vegetable kingdom.

1. The precipitate from a 10% NaCl extract by *saturation with magnesium sulphate* in *neutral* solution will consist of,

Globulins of the myosin and paraglobulin type.

β-phytalbumose of the character above described.

In *acid* solution,

Also of *a-phytalbumose* (vegetable 'peptone,' Vines' hemialbumose).

2. The precipitate in *neutral* solution by *saturation with sodium sulphate after saturation with magnesium sulphate* will consist of,

vegetable albumin,

vegetable vitellin,

a-phytalbumose.

The vitellin may be separated from the albumen by dialysis, being precipitated; or by dilution of the liquid and the passage of a current of CO_2 through it for some hours.

Fractional precipitation by heat.

The method just described is also usefully supplemented by a fractional precipitation by heat, which was performed with the following results in a neutralised 10% NaCl extract of the juice.

The liquid became cloudy at 70° C., the cloudiness increasing to 74° C., at which point it settled to a fine precipitate in a short time. The liquid was filtered. The filtrate, heated again, became cloudy at 78° C., the cloudiness increasing to 82° C., at which temperature a precipitate fell, soluble in 2% H^2SO_4. The precipitate being removed, the filtrate became cloudy again at 83° C., increasing to 92° C.; the liquid raised to 100° deposited a fine precipitate, which was soluble in ·2% KHO. The liquid being filtered, the filtrate gave a precipitate with nitric acid, mostly soluble on boiling, coming down again on cooling etc.

[1] This *Journal*, Vol. v.

From what we have previously seen the first precipitate was the globulin; the second and third β-phytalbumose precipitable by heat; and in the last filtrate was the α-phytalbumose.

Leucin and Tyrosin in the juice.

The juice extracted with boiling absolute alcohol and filtered, deposited impure crystals of leucin and tyrosin: it contained no proteid, but gave Scherer's test for leucin and Hofmann's for tyrosin (Millon's reagent).

Leucin is in much greater abundance than tyrosin.

A summary may now be given of the proteids present in the juice examined.

1. *Globulin,* soluble like other bodies of its class only in saline solutions, precipitated from these by saturation with sodium chloride and magnesium sulphate, by dialysis, and by diluting many times and passing carbonic acid through the liquid. Coagulation temperature in 10%, NaCl solution 70°—74° C.

Weyl[1] has described two forms of globulin as occurring in plants, which he calls vegetable myosin and vegetable vitellin, the former precipitated by saturation with sodium chloride and coagulated by heat (in 10% NaCl) at 55°—60° C.; the latter not precipitated by saturation with sodium chloride, and coagulating at 73° C. in 10% NaCl solution.

The globulin described above agrees with vegetable myosin in all properties except the coagulation temperature, which is much higher, 70° C.—73° C., this being indeed nearly the temperature Weyl gives for the coagulation point of vitellin. However the globulin differs from vitellin in being precipitated by saturation with sodium chloride. It corresponds indeed neither to plant-myosin nor to plant-vitellin: it is more related to paraglobulin than to any other animal globulin.

2. An albumin, soluble in water, not precipitated by saturation with neutral salts, but coagulated by heat.

3. Two forms of albumose.

(*a*) *β-phytalbumose* precipitated almost completely by heat, in two stages at from 78°—82° C., and from 83°—95° C. This body also is precipitated by saturation with sodium chloride and magnesium sulphate, like the globulin, but is not thrown down by dialysis. It corresponds in its reaction with the body described by Kühne and Chit-

[1] *Zeits. für physiolog. Chemie*, 1877–78, Bd. 1, S. 72.

tenden as hetero-albumose, except that it is not precipitated by dialysis, by copper sulphate nor by mercuric chloride.

(*b*) The other form *a-phytalbumose* is soluble in cold or boiling water, and is not precipitated by saturation with NaCl or $MgSO_4$ in *neutral* solution: it however comes down after prolonged shaking with either salt in *acid* solution.

This body is the vegetable 'peptone' referred to by Vines as hemialbumose. It agrees with Kühne and Chittenden's protalbumose in all points except its non-precipitation by saturation with sodium chloride in neutral solution and its non-precipitation by copper sulphate.

Both albumoses give a biuret reaction: β-phytalbumose a very faint one, α-phytalbumose a well-marked one.

4. No peptones were found in the juice.
5. Leucin and tyrosin are present.

Proteolytic changes in the proteids.

Having thus shown the presence of these proteids, the question arose as to what change they underwent in artificial digestion by papaïn, as the results obtained might be of aid in ascertaining the proteolytic changes in the plant itself.

It has been said by Vines[1] that "it is remarkable that the reserve-proteids of plants should consist of substances which find near allies in the products of the digestion, more particularly the pancreatic, of proteids by animals."

As opposed to this, however, it may be said that nearly all the proteolytic ferments that have as yet been separated from plants have been proved to act like pepsin in an acid solution. Such is the ferment extracted by Gorup-Besanez[2] from vetches, in which it is associated with a diastasic ferment; of a similar character is the ferment extracted by Krukenberg[3] from the plasmodium of *Aethalium septicum*, one of the Mycomycetes. Both these ferments act on animal proteid in ·2% hydric chloride, forming peptones. The ferments produced by the carnivorous plants have been proved by Darwin, Gorup-Besanez, Will and others to act only in an acid medium. That

[1] This *Journal*, Vol. III. p. 113.

[2] *Ber. d. deutsch. chem. Gesell.* VII. 1874. Vide also *ibid.*, viii., 1875, p. 1510: in which similar ferments from the seeds of hemp, flax, and malt are described.

[3] *Untersuch. aus dem physiolog. Institute der Univ. Heidelberg*: Band. II. Hft. 3, 1878.

extracted by Bouchut[1] from the fruit of the fig, *Ficus carica*, acts, according to Hansen[2], best in an acid medium, not so well in one made alkaline with sodium carbonate: this ferment also curdles milk at 50° C.

Of these ferments, it will be seen that the one occurring in the fig most resembles papaïn; not only in the curdling of milk but in the fact that it is capable of acting in a slightly alkaline medium, though not so energetically as in an acid one.

More definite knowledge is certainly requisite concerning the action of all vegetable proteolytic ferments: and it is necessary, in order to ascertain their rôle in the economy of plants, to test their action on the proteids occurring in association with them, which are usually the reserve proteids of the seeds and fruit. This has been done with papaïn in the experiments about to be recorded.

It is first, however, essential that we should ascertain the distribution of a true peptone in plants. All observers have tested the action of the proteolytic ferments on animal proteid (fibrin, egg-albumin) and all are agreed that peptones are produced; in some cases hemialbumose as well[3].

As we have before stated, no true peptone is found in the papaw juice: and Vines states that the body called by previous observers 'vegetable peptone' is hemialbumose, Meissner's *a*-peptone. Do these vegetable proteolytic ferments, then, form peptones from their associated proteids? It is quite possible, *à priori*, that they do, the peptone having as short-lived an existence, as such, as in animal digestion, and undergoing a rapid reconstruction into utilisable proteid.

Such questions as these suggested the experiments about to be recorded.

We have seen that there are present in papaw juice, a globulin, an albumin, and two albumoses, one precipitated by heat, the other not, leucin and tyrosin being also present. The action of papaïn on each of these proteids was tested.

In all cases the digestive mixture was neutral, since the fresh juice itself is neutral[4]. Both the watery and saline extracts of the dried juice used was acid, but this is due to decomposition, acid albumin being formed. Control flasks without the ferment were in all cases prepared, so as to compare the action, and thymol was added to prevent decomposition, which it effectually did.

[1] *Compt. Rend*, xci. Juillet, 1880.

[2] *Biologisches Centralblatt*, 1881.

[3] Krukenberg, *op. cit.*

[4] Wurtz, *Compt. Rend.*, 1880.

Action of papaïn on a mixture of the globulin and two albumoses.

The magnesium sulphate precipitate of the acid 15% NaCl extract of the juice was dissolved and dialysed till nearly all the salt was removed: the precipitated globulin was then redissolved by adding a small quantity of the salt. This solution then contained a large quantity of globulin, about an equal quantity of β-phytalbumose, and a little α-phytalbumose: saturation with magnesium sulphate in neutral solution gave a dense precipitate.

After dilution with water, 1 gramme of papaïn dissolved and filtered was added, and the neutral mixture incubated at 33°—35° C. for 22½ hours.

There was a slight precipitate at the bottom of the flask, which was filtered off. The filtrate gave only a marked cloudiness on boiling, very different from the dense precipitate obtained before digestion, showing that the proteids had undergone some change. Saturation of a portion of the filtrate with magnesium sulphate caused only a slight precipitate, so little that no correct examination of its character was possible. This showed that most of the globulin had disappeared. The precipitate was filtered off and the filtrate saturated with sodium sulphate, and then shaken with sodio-magnesium sulphate to complete the precipitation of all the remainder of the precipitable proteid. This saturation caused a fairly dense precipitate, which was dissolved in water (after washing with a saturated solution of the double salt) and dialysed till there was only a trace of sulphates and chlorides present: dialysis made the liquid slightly turbid: the liquid was filtered till clear. The filtrate gave a precipitate on boiling, insoluble in 10% magnesium sulphate, but soluble in dilute acid and alkali: in fact consisting of β-phytalbumose. Nitric acid also gave a precipitate, behaving like an albumose, the presence of which was further demonstrated by the solution giving all the reactions characteristic of the body. The filtrate after boiling gave only the faintest xanthoprotein reaction, showing that most, if not all, of the α-phytalbumose had disappeared.

On boiling the original digestive filtrate with ferric acetate (in the manner previously detailed) and filtering, it was found that the filtrate gave no biuret reaction, no precipitate with nitric acid, or with acetic acid and potassic ferrocyanide, but an opalescence with acetic acid and metatungstic acid, showing the presence of a mere trace of peptones. Leucin and tyrosin were also found in this filtrate.

This experiment therefore shows, that by the digestion of a mixture of globulin, *a*-phytalbumose and *β*-phytalbumose with a large quantity of papaïn in neutral solution, the globulin almost entirely disappears, the *a*-phytalbumose completely so; the chief proteid in solution being *β*-phytalbumose, a slight trace of peptone being present. From the absence of *a*-phytalbumose, it may be presumed that the globulin has become changed partly into the other albumose, partly into peptone, which has split up into leucin and tyrosin. Again, the absence of *a*-phytalbumose may be explained by a change into peptone, and then into leucin and tyrosin. The presence of the large quantity of *β*-phytalbumose may be explained either by considering that the quantity of ferment was insufficient to produce any further change, or that it is an anti-product, and incapable of further change by the ferment: both causes, of course, might act. This was therefore tested by digesting with a fresh quantity of ferment.

Action of papaïn on β-phytalbumose.

The solution of *β*-phytalbumose was digested at 44°—45° C. for 22½ hours with a small quantity of the glycerine solution of purified papaïn (previously described): an experiment without the ferment being performed at the same time. After digestion, there was a precipitate in the liquid (? the anti-residue), slightly soluble, but with great difficulty, in 10% sodium chloride solution, from which it was precipitated by nitric acid. After filtering the digestive liquid, boiling gave only a marked cloudiness, mostly soluble in dilute acid, and a similar cloudiness with nitric acid, soluble on boiling. As compared with the liquid incubated without the ferment, the difference was marked, the latter giving a dense precipitate with boiling and nitric acid. Further, after boiling and filtering, the liquid gave only a slight opalescence on boiling, no cloudiness with nitric acid, but a marked xanthoprotein reaction, a biuret reaction, and a fine precipitate with acetic acid and potassic ferrocyanide: saturation with sodio-magnesium sulphate also caused a precipitate. Most of the albumose had then disappeared, and in its place was a body which is found in the filtered liquid after boiling. This body is not a true peptone, since it is precipitated by acetic acid and potassic ferrocyanide, and by saturation with the double salt: it is not *a*-phytalbumose, since it is not thrown down by nitric acid. It is, moreover, not precipitated by boiling with ferric acetate, nor by saturation with sodium chloride or magnesium sulphate alone: but basic acetate of lead gives a precipitate

soluble in excess, and mercuric chloride gives a cloudiness. The exact relations of this body I am at present unable to state: it resembles most nearly Meissner's *b*-peptone; it is also like Kühne and Chittenden's deutero-albumose[1], but it differs in the fact that a solution made slightly acid with acetic acid does not become, like deutero-albumose, cloudy on warming. These two bodies, deutero-albumose and *b*-peptone, bear such a close resemblance to each other that it is rather like splitting a hair to try and distinguish them.

However this may be, the experiment shews the transformation of a greater part of the *β*-phytalbumose into a peptone-like body: leucin and tyrosin are here also bye-products.

Action of the ferment on a-phytalbumose.

A solution of this body was prepared by boiling an aqueous extract of the juice (after neutralisation), and filtering till quite clear. This liquid gave the reactions previously described as characteristic of the body: the chief of which are that there was no precipitate on boiling, but a dense one with nitric acid.

Digestion in neutral solution with the glycerine extract of the purified papaïn was continued for 23 hours at 40°—45° C. After digestion there was a slight flocculent deposit in the liquid which was filtered off. The clear filtrate gave no precipitate on boiling, and only a slight cloudiness on adding nitric acid, showing the disappearance of most of the albumose. The liquid moreover gave marked xantho-protein and biuret reactions, and a precipitate with acetic acid and potassic ferrocyanide. Boiled with ferric acetate, the filtrate gave no cloudiness with nitric acid (showing the absence of *a*-phytalbumose), but a precipitate with acetic acid and potassic ferrocyanide, and acetic acid and metatungstic acid: also a biuret reaction. Further boiling of the filtrate with ferric acetate did not remove the body giving a precipitate with acetic acid and potassic ferrocyanide. Saturation with sodio-magnesium sulphate caused a precipitate. We have then evidence of the formation from *a*-phytalbumose of the same body which is a product of digestion of *β*-phytalbumose. Many experiments confirmed both these results: there was no evidence of a true peptone being formed.

Leucin and tyrosin are also formed.

[1] *Op. cit.* p. 28.

Action on the albumin.

A solution of the albumin was prepared as before. It contained a trace of *a*- but no *β*-phytalbumose: the precipitate on boiling was wholly coagulated proteid.

The solution was digested with purified papaïn for 27½ hours at 42° C. After digestion, there was a slight deposit in the liquid which was filtered. The filtrate gave a precipitate on boiling, mostly soluble in ·2% KHO, and a precipitate with nitric acid, mostly soluble on boiling, and behaving like an albumose. The filtrate after boiling gave a cloudiness with nitric acid, and a fairly well-marked xantho-protein reaction: no biuret-reaction with copper-sulphate and excess of potash.

A great part of the albumin had then been changed into an albumose, chiefly *β*-phytalbumose, since saturation with sodium chloride in neutral solution caused a dense precipitate.

I have not investigated the further changes of the albumin, as the stock of material was exhausted.

From this series of digestive experiments, we see that the globulin undergoes a change into *β*-phytalbumose and that this becomes a peptone-like body and forms leucin and tyrosin. The albumin also undergoes a change into the same albumose. The *a*-phytalbumose becomes a *b*-peptone and splits up into leucin and tyrosin.

Such changes probably go on in the plant itself, though too much stress must not be laid on the point. The artificial digestion was performed in a much greater excess of liquid than is present in the juice itself: so that the slow digestion occurring above would be still slower in the plant. This very slow proteolysis would limit the formation of the peptone-like body, and thus the production of leucin and tyrosin; for we know of no other mode of formation of these two bodies by a proteolytic ferment, but by the splitting up of a peptone.

To what extent this peptone is formed in the plant, and further how much of it formed is split up into leucin and tyrosin must be left unsettled. The small proportion of it present under the most advantageous conditions of artificial digestion would point perhaps to the fact that it is not a proteid which circulates.

The proteids which then would circulate would be the albumoses, as being more soluble than the globulin or albumin: and this supposition would agree with an observation of Vines, in which he found hemi-albumose in quantity in the cotyledons of germinating *Lupinus hirsutus*, and none in the seedlings, only asparagine.

Extended observations are necessary as to the proteolytic changes in the reserve proteids of plants. It is not sufficient to test the action of the ferments found on animal proteids; we must extend the experiments to the proteids with which the ferment is associated.

It is evident moreover that too general a deduction cannot at present be drawn as to the nature of the proteolytic change, as to whether the agent acts like animal pepsin or like trypsin. Many observers, some of whom have been quoted, have shewn the existence of vegetable ferments like pepsin: papaïn however is the only one which has as yet been proved to act like trypsin, and then its normal action takes place in a neutral medium. The ferment in *Ficus carica* seems to resemble it but as the juice of the unripe fig is acid, the resemblance cannot be very marked. Why, indeed, there should be a pepsin-like ferment in some plants and a trypsin-like in others is as much a problem as why there should be the two forms in mammals. Experiments and observations are as yet too scanty to draw any conclusions as to the evolution of ferment-processes in plants and the lower animals: a wide field is still open.

I cannot conclude without expressing my indebtedness to the classical researches of Kühne and Chittenden, which have thrown so much light on the processes of digestion in animals.